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CATHELICIDIN LL-37: LPS-NEUTRALIZING, PLEIOTROPIC PEPTIDE

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Abstract: Human organism, constantly exposed to a large variety of pathogenic microorganisms and their products, such as lipopolysaccharide (LPS), developed innate immunity as a first line of defence. One of the compartments of our organism well equipped with these defence mechanisms is the respiratory system. The cells lining the airways respond to the presence of virulent microorganisms by producing natural antimicrobial peptides, including the only member of the cathelicidins family found to date in humans, peptide LL-37. LL-37 is a small peptide of 37 amino acid residues. The peptide, in addition to its bactericidal effect, plays numerous roles in inflammatory and tissue remodelling processes. It stimulates angiogenesis, induces proliferation of lung epithelial cells, accelerates wound closure of the airway epithelium, and provokes cytokine release (e.g. IL-8) and cell migration. LL-37 is also able to neutralize LPS, a heteropolymer associated with organic dust, produced by Gram-negative bacteria. LPS (commonly referred to as endotoxin) plays an important role in pathogenesis of many respiratory diseases caused by organic dust, including organic dust toxic syndrome and chronic illnesses such as chronic obstructive pulmonary disease (COPD), asthma or allergic alveolitis (hypersensitivity pneumonitis). LPS is a strong pro-inflammatory stimulus, inducing in respiratory airways expression of antimicrobial peptides, including LL-37, which is in turn a potent LPS-neutralizing factor. The article discusses the complex interplay between endotoxin and the LPS-neutralizing, pleiotropic peptide LL-37 in pathogenic mechanisms of lung diseases, with regard to closer perspectives of using LL-37 and its derivatives as therapeutic agents.

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INTRODUCTION

The human organism, constantly exposed to a large variety of pathogenic microorganisms and their products, such as endotoxin, has developed innate immunity as a first line of defence. One of the compartments of our organism well equipped with these defence mechanisms is the respiratory system, being constantly exposed to airborne bacteria, fungi, viruses and other pollutants including dusts and volatile compounds. The respiratory epithelium is an important interface with the environment and represents a key system with regard to the innate host immunity of the lung. In addition to major immunoregulatory properties [15, 21], the cells lining the airways respond to the presence of virulent microorganisms by producing natural antimicrobial peptides (AMPs). These factors play also numerous roles in many processes preserving homeostasis in the airways [4]. These antimicrobial peptides (AMPs) provide immediate protection against the invasion of a broad spectrum of microorganisms, including Gram-negative bacteria releasing

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to the environment lipopolysaccharides (LPS) – heteropolymeric components of their outer layer membrane with strong immunotoxic properties.

ANTIMICROBIAL PEPTIDES – HUMAN CATHELICIDIN: PEPTIDE LL-37

The first descriptions of this group of peptides appeared in early 70s of the past century. During the last three decades, hundreds of antimicrobial peptides have been discovered, both in plants and animals. In humans, these peptides include members of three antimicrobial peptide families: defensins, histatins and cathelicidins [28]. Cathelicidins have been found in cows (BMAP-27, indolicidin, and bactenecin), pigs (protegrins), mice (CRAMP), rabbits (CAP18) [2]. This family of peptides derives from proteins that contain a highly conserved amino acid sequence and a proregion highly homologous to cathelin, a cathepsin L inhibitor. However, cathelicidins' C-terminal domain shows substantial heterogeneity [28].

The only member of the cathelicidins family found to date in humans is LL-37/hCAP18, an 18 kDa peptide encoded by the gene *CAMP*, which was first described in humans in 1995 in bone marrow cells. LL-37/hCAP18 is a small peptide of 37 amino acid residues starting with two leucine residues [23]. This peptide is derived by extracellular proteolysis from the C-terminal end of the human CAP18 protein (hCAP18) [28]. LL-37 has been found in leukocytes and epithelial cells of the airways, but also within the gastrointestinal and urinary tracts and the skin. LL-37 is also likely to be involved in the pathogenesis of several diseases (e.g. morbus Kostmann, a severe congenital form of neutropenia, originally defined in descendants of the Kostmann family characterized by lacking antimicrobial peptide LL-37) [23]. LL-37 is a part of our immune system which is present in the human organism at a very early stage of development – the peptide has been found not only in newborns but even in amniotic fluid [33]. The role of LL-37 has been also studied in pulmonary pathologies – e.g. in sarcoidosis, where activation of antibacterial defence system has been suggested [1].

LL-37 is actively secreted into the airway surface fluid layer where it can be found at concentrations of an average of about 2 μl/ml [3, 12, 20, 30] and exerts a broad antibacterial activity [28] typical for cathelicidins. LL-37 performs its bactericidal action by electrostatic binding of its cationic molecules to the outer surface of the bacterial cell. Insertion of the peptide into the cell membrane results in leakage of the cell cytoplasm into the extracellular space causing death of the bacterial cell [28]. In addition to its bactericidal effect, LL-37 is able to neutralize LPS, and in doing so, protects against endotoxic shock [10, 28]. LL-37 binds to LPS, dissociates endotoxin aggregates and competes with LPS on the binding site within the CD14 receptor, preventing endotoxin-dependent cytokine induction and macrophage activation [24]. This activity has been confirmed by both in *in vitro* [24] and *in vivo* studies [10]. *In vitro* studies also showed that LL-37 prevented the proinflammatory activation of monocytes due to LPS by suppressing Toll-like receptor (TLR)-induced secretion of proinflammatory cytokines [16]. In addition to its direct antimicrobial and anti-LPS functions, LL-37 interacts also with human cells by the formyl peptide receptor-like 1 (FPRL1) [32] and the P2X7 receptor [7]. This enables pleiotropic role of the peptide in inflammation and tissue repair processes, influencing correct functioning of the respiratory tract. LL-37 stimulates angiogenesis [13], induces proliferation of lung epithelial cells [26], accelerates wound closure of the airway epithelium [26], and provokes cytokine release (e.g. IL-8) and cell migration [13]. Human cells exposed to LL-37 can undergo necrosis [26].

Extensive clinical use of antibiotic drugs has led to a dramatic increase of the resistance of various microorganisms, especially within the lung. As a result, development of new classes of antibiotics has become one of the priorities for biomedical sciences and the pharmaceutical industry [17]. Antimicrobial peptides and their synthetic derivatives are seen as a potential alternative for currently used antibiotics. This is further encouraged by the improvement of wound healing caused by antimicrobial peptides.

LPS AND LL-37: INVOLVEMENT IN PATHOGENESIS OF RESPIRATORY DISEASES

A variety of polysaccharides produced by Gram-negative bacteria play an important role in pathogenesis of many exogenous respiratory diseases, including organic dust toxic syndrome and chronic illnesses such as COPD, asthma or allergic alveolitis (hypersensitivity pneumonitis). LPS adds also to the course of Gram-negative infections and its massive release can lead to life threatening pathology: endotoxic shock [25]. Endotoxins stimulate human organism immune system response and cause injury to the respiratory epithelia [19, 25] – which results in enhancing inflammation and tissue repair. Millions of people working in agriculture and related industries (e.g. herb and wood processing facilities) are chronically exposed to LPS [8, 9, 22]. Apart from its mostly pathogenic role, moderate long-term exposure to LPS has been also described as a protective factor against lung cancer [14] and asthma in countryside children [27].

LPS, as a strong pro-inflammatory stimulus, induces in respiratory airways expression of antimicrobial peptides, including LL-37, which is in turn a potent LPS-neutralizing factor [5, 18]. By this activity LL-37 is protecting airways against LPS-derived activation of immune system. It is also conceivable that the peptide LL-37, due to the wide spectrum and character of its biological functions (including enhancing inflammatory reaction, tissue remodeling processes and its antimicrobial and anti-LPS activities), also takes part in the pathogenesis of chronic inflammatory diseases of the respiratory system, such as

COPD. As a result of this, LL-37 may exert both beneficial and pathological effects during the development of chronic inflammatory lung diseases. An ambivalent role of LL-37 is possible, especially in the case of lung diseases caused by organic dust – in the presence of long-term and recurrent exposure to LPS – and lung injuries caused by inhaled organic dust particles.

Xiao *et al.* [31] have observed higher levels of LL-37 in the sputum of patients with COPD and reduced concentration of LL-37 in asthma patients compared to healthy volunteers. This may suggest a role of LL-37 in the development of COPD and asthma.

These data may indicate a versatile, complex interplay between endotoxin and the LPS-neutralizing, pleiotropic peptide LL-37 in pathogenic mechanisms of lung diseases. Potentially, the nature of these interactions differs depending on the kind (asthma/COPD) and character of disease – either chronic (COPD, asthma) – or acute (endotoxic shock, infectious exacerbations in chronic diseases, organic dust toxic syndrome).

CONCLUSIONS

LL-37 and its synthetic derivatives are under investigation as therapeutic agents – pulmonary diseases are of high interest for such LL-37-based therapeutic strategies, which can potentially use combined anti-LPS, antimicrobial and wound healing features of the peptide [17]. Immunostimulating effects of endotoxin are also in the scope of scientists´ interests due to its expected beneficial effects if properly administered [6, 29, 11]. Thus, there is an important need of further unraveling the role of LL-37, including especially LL-37/LPS interactions, in pulmonary pathologies. This is highly requested in agricultural environment, where exposure to airborne LPS and diseases caused by organic dust are very common.

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